

Listing of Claims:

C / Claims 1-11 (Canceled)

1 12. (Previously Amended) A commercial-scale method of sialylating a
2 saccharide group on a recombinant glycoprotein, the method comprising contacting a saccharide
3 group which comprises a galactose or N-acetylgalactosamine acceptor moiety on a recombinant
4 glycoprotein with a sialic acid donor moiety and a recombinant bacterial sialyltransferase in a
5 reaction mixture which provides reactants required for sialyltransferase activity for a sufficient
6 time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to
7 said saccharide group.

1 13. (Original) The method of claim 12, wherein the bacterial sialyltransferase
2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Neisseria meningitidis* 2,3-sialyltransferase.

1 14. (Original) The method of claim 13, wherein the bacterial sialyltransferase is
2 a *Neisseria meningitidis* 2,3-sialyltransferase.

1 15. (Original) The method of claim 12, wherein the bacterial sialyltransferase
2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Photobacterium damsela* 2,6-sialyltransferase.

1 16. (Original) The method of claim 15, wherein the bacterial sialyltransferase is
2 a *Photobacterium damsela* 2,6-sialyltransferase.

1 17. (Original) The method of claim 12, wherein the bacterial sialyltransferase
2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Haemophilus* 2,3-sialyltransferase.

1 18. (Original) The method of claim 17, wherein the sialyltransferase is a

2 *Haemophilus* 2,3-sialyltransferase.

1 19. (Original) The method of claim 12, wherein the bacterial sialyltransferase

2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a

3 *Campylobacter jejuni* 2,3-sialyltransferase.

1 20. (Original) The method of claim 19, wherein the sialyltransferase is a

2 *Campylobacter jejuni* 2,3-sialyltransferase.

1 21-22. (Cancelled)

1 23. (Previously Amended) A commercial-scale method of sialylating a

2 saccharide group on a recombinant glycoprotein, the method comprising contacting a saccharide

3 group which comprises a galactose or an N-acetylgalactosamine acceptor moiety on a

4 recombinant glycoprotein with a sialic acid donor moiety and a bacterial sialyltransferase in a

5 reaction mixture which provides reactants required for sialyltransferase activity for a sufficient

6 time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to

7 said saccharide.

1 24. (Original) The method of claim 23, wherein the bacterial sialyltransferase

2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a

3 *Photobacterium damsela* 2,6-sialyltransferase.

1 25. (Original) The method of claim 24, wherein the bacterial sialyltransferase is

2 a *Photobacterium damsela* 2,6-sialyltransferase.

1 26. (Original) The method of claim 22, wherein the bacterial sialyltransferase
2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Neisseria meningitidis* 2,3-sialyltransferase.

1 27. (Original) The method of claim 26, wherein the sialyltransferase is a
2 *Neisseria meningitidis* 2,3-sialyltransferase.

1 28. (Original) The method of claim 23, wherein the bacterial sialyltransferase
2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Campylobacter jejuni* 2,3-sialyltransferase.

1 29. (Original) The method of claim 28, wherein the sialyltransferase is a
2 *Campylobacter jejuni* 2,3-sialyltransferase.

1 30. (Original) The method of claim 23, wherein the bacterial sialyltransferase
2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Haemophilus* 2,3-sialyltransferase.

1 31. (Original) The method of claim 30, wherein the sialyltransferase is a
2 *Haemophilus* 2,3-sialyltransferase.

32-43. (Canceled)

1 44. (Previously amended) A commercial-scale method for *in vitro* sialylation of
2 saccharide groups on a glycoprotein, said method comprising contacting said saccharide groups
3 with a sialyltransferase, wherein the sialyltransferase is a bacterial sialyltransferase, a sialic acid
4 donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and

5 under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said
6 saccharide group.

1 45. (Original) The method of claim 44, wherein the bacterial sialyltransferase is
2 a recombinant sialyltransferase.

1 46. (Original) The method of claim 44, wherein the bacterial sialyltransferase
2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Neisseria meningitidis* 2,3-sialyltransferase.

1 47. (Original) The method of claim 46, wherein the bacterial sialyltransferase is
2 a *Neisseria meningitidis* 2,3-sialyltransferase.

1 48. (Original) The method of claim 44, wherein the bacterial sialyltransferase
2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Photobacterium damsela* 2,6-sialyltransferase.

1 49. (Original) The method of claim 48, wherein the bacterial sialyltransferase is
2 a *Photobacterium damsela* 2,6-sialyltransferase.

1 50. (Original) The method of claim 44, wherein the bacterial sialyltransferase
2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Campylobacter jejuni* 2,3-sialyltransferase.

1 51. (Original) The method of claim 50, wherein the sialyltransferase is a
2 *Campylobacter jejuni* 2,3-sialyltransferase.

1 52. (Original) The method of claim 44, wherein the bacterial sialyltransferase
2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Haemophilus* 2,3-sialyltransferase.

1 53. (Original) The method of claim 52, wherein the sialyltransferase is a
2 *Haemophilus* 2,3-sialyltransferase.

1 54. (Canceled)

1 55. (Original) The method of claim 54, wherein the CMP-sialic acid is
2 enzymatically generated *in situ*.

1 56. (Original) The method of claim 32, wherein the sialic acid is selected from
2 the group consisting of NeuAc and NeuGc.

1 57. (Previously amended) A commercial-scale method for *in vitro* sialylation of
2 terminal galactose residues on a glycoprotein, said method comprising contacting said
3 glycoprotein with a reaction mixture that comprises a sialyltransferase, a sialic acid donor
4 moiety, and other reactants required for sialyltransferase activity, for a sufficient time and under
5 appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said terminal
6 galactose residues.

1 58. (Original) The method of claim 57, wherein the method further comprises
2 contacting the saccharide groups with an ST6GalI sialyltransferase.

1 59. (Previously added) A method for *in vitro* sialylation of terminal galactose
2 residues present on a glycoprotein, said method comprising contacting said glycoprotein with a
3 reaction mixture that comprises a sialyltransferase, wherein the sialyltransferase is a bacterial

4 sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase
5 activity, for a sufficient time and under appropriate conditions to transfer sialic acid from said
6 sialic acid donor moiety to said terminal galactose residues, wherein a greater percentage of
7 terminal galactose residues are sialylated compared to an unaltered glycoprotein.

1 60. (Previously added) The method of claim 59, wherein at least 80% of the
2 terminal galactose residues present on the glycoprotein are sialylated.

1 61. (Previously added) The method of claim 60, wherein at least 90% of the
2 terminal galactose residues present on the glycoprotein are sialylated.

1 62. (Previously added) The method of claim 59, wherein the terminal galactose
2 residues comprise one or more saccharides selected from the group consisting of
3 Gal β 1,4GlcNAc, Gal β 1,4GalNAc, Gal β 1,3GalNAc, Gal β 1,3GlcNAc, Gal β 1,3Ara,
4 Gal β 1,6GlcNAc, and Gal β 1,4Glc.

1 63. (Previously added) The method of claim 62, wherein the terminal galactose
2 residues comprise Gal β 1,4GlcNAc or Gal β 1,3GlcNAc.

1 64. (Previously added) The method of claim 63, wherein at least 80% of the
2 terminal Gal β 1,4GlcNAc residues present on the glycoprotein are sialylated.

1 65. (Previously added) The method of claim 63, wherein at least 80% of the
2 terminal Gal β 1,3GlcNAc residues present on the glycoprotein are sialylated.

1 66. (Previously added) The method of claim 59, wherein the terminal galactose
2 residues are present on an O-linked oligosaccharide.

1 67. (Previously added) The method of claim 59, wherein the terminal galactose
2 residues are present on an N-linked oligosaccharide.

1 68. (Previously added) The method of claim 59, wherein the sialyltransferase
2 includes a sialyl motif which has an amino acid sequence that is at least about 40% identical to a
3 sialyl motif from a sialyltransferase selected from the group consisting of ST3Gal I, ST6Gal I,
4 and ST3Gal III.

1 69. (Previously added) The method of claim 68, wherein the sialyltransferase is
2 an ST3Gal III.

1 70. (Previously added) The method of claim 69, wherein the sialyltransferase is
2 a rat ST3Gal III.

1 71. (Previously added) The method of claim 68, wherein the sialyltransferase is
2 an ST3Gal IV.

1 72. (Previously added) The method of claim 68, wherein the sialyltransferase is
2 an ST6Gal I.

1 73. (Previously added) The method of claim 68, wherein the sialyltransferase is
2 an ST3Gal I.

1 74. (Previously added) The method of claim 59, wherein the bacterial
2 sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid
3 sequence of a *Neisseria meningitidis* 2,3-sialyltransferase.

1 75. (Previously added) The method of claim 74, wherein the bacterial
2 sialyltransferase is a *Neisseria meningitidis* 2,3-sialyltransferase.

1 76. (Previously added) The method of claim 73, wherein the bacterial
2 sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid
3 sequence of a *Photobacterium damsela* 2,6-sialyltransferase.

1 77. (Previously added) The method of claim 76, wherein the bacterial
2 sialyltransferase is a *Photobacterium damsela* 2,6-sialyltransferase.

1 78. (Previously added) The method of claim 59, wherein the bacterial
2 sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid
3 sequence of a *Haemophilus* 2,3-sialyltransferase.

1 79. (Previously added) The method of claim 78, wherein the sialyltransferase
2 is a *Haemophilus* 2,3-sialyltransferase.

1 80. (Previously added) The method of claim 59, wherein the bacterial
2 sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid
3 sequence of a *Campylobacter jejuni* 2,3-sialyltransferase.

1 81. (Previously added) The method of claim 80, wherein the sialyltransferase
2 is a *Campylobacter jejuni* 2,3-sialyltransferase.

1 82. (Previously amended) A commercial-scale method for altering the
2 glycosylation pattern of a glycoprotein *in vitro*, the method comprising contacting a
3 glycoprotein-linked saccharide with a galactosyltransferase in the presence of UDP-galactose
4 under suitable conditions for the galactosyltransferase to transfer a galactose residue from the
5 UDP-galactose to the saccharide to form a galactosylated saccharide.

83-97. (Canceled)

1 98. (Previously added) The method of claim 12, wherein the glycoprotein
2 comprises an immunoglobulin.

1 99. (Previously added) The method of claim 23, wherein the glycoprotein
2 comprises an immunoglobulin.

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1 100. (Previously added) The method of claim 44, wherein the glycoprotein
2 comprises an immunoglobulin.

1 101. (Previously added) The method of claim 57, wherein the glycoprotein
2 comprises an immunoglobulin.

1 102. (Previously added) The method of claim 82, wherein the glycoprotein
2 comprises an immunoglobulin.

1 103. (New) A method for *in vitro* sialylation of a saccharide group present on a
2 glycoprotein, said method comprising:
3 (a) modifying said glycoprotein to create an acceptor; and
4 (b) sialylating said acceptor formed in (a) with a sialyltransferase in the presence
5 of a CMP derivative of a sialic acid using an $\alpha(2,3)$ sialyltransferase under conditions in which
6 sialic acid is transferred to a non-reducing sugar present on said glycoprotein.

1 104. (New) The method according to claim 103, wherein said modifying
2 comprises:

3 galactosylating a compound of the formula $\text{GlcNR}'\beta(1 \rightarrow 3)\text{Gal}\beta\text{-OR}$ with a
4 galactosyltransferase in the presence of a UDP-galactose under conditions sufficient to form
5 $\text{Gal}\beta(1 \rightarrow 4)\text{GlcNR}'\beta(1 \rightarrow 3)\text{Gal}\beta\text{-OR}$, wherein:

6 R is a member selected from the group consisting of an amino acid, a saccharide,
7 an oligosaccharide, and an aglycon group having at least one carbon; and wherein:

8 R' is a member selected from the group consisting of acetyl and allyloxycarbonyl.

1 105. (New) The method according to claim 104, wherein R is linked to or is
2 part of a glycoprotein.

Coh 1 106. (New) The method according to claim 104, wherein said galactosylating
C 2 and sialylating are carried out enzymatically.

1 107. (New) The method according to claim 104, wherein said galactosylating
2 is carried out as part of a galactosyltransferase cycle.

1 108. (New) The method according to claim 104, wherein said sialylating is
2 carried out as part of a sialyltransferase cycle.

1 109. (New) The method according to claim 104, wherein said method
2 comprises carrying out said galactosylating and said sialylating in a single reaction mixture that
3 contains both a sialyltransferase and a galactosyltransferase.

1 110. (New) The method according to claim 109, wherein said sialyltransferase,
2 said galactosyltransferase, and said GlcNR'β(1→3)Galβ-OR are combined in an initial reaction
3 mixture.

1 111. (New) The method according to claim 109, wherein said method further
2 comprises the addition of said sialyltransferase, said galactosyltransferase, and said
3 GlcNR'β(1→3)Galβ-OR for a second glycosyltransferase cycle once a first glycosyltransferase
4 cycle has neared completion.
